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RESULTS OF A PILOT TEST FOR THE PRESENCE OF VITELLOGENINS IN MUSCLE TISSUE OF SWORDFISH (*XIPHIAS GLADIUS*) SAMPLED AT THE HONOLULU FISH AUCTION

Edward E. DeMartini

Honolulu Laboratory
Southwest Fisheries Science Center
National Marine Fisheries Service, NOAA
2570 Dole Street
Honolulu, Hawaii 96822-2396

and

Jennifer Specker

Graduate School of Oceanography
University of Rhode Island
Narragansett, Rhode Island 02882-1197

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ABSTRACT

A polyclonal antibody assay, previously developed for detecting vitellogenins (VTG, sex-specific protein precursors to oocyte yolk) present in the tissues of a percoid teleost (tilapia, *Oreochromis mossambicus*), was applied to muscle tissue specimens collected from commercially caught swordfish (*Xiphias gladius*) carcasses sampled at the Honolulu fish auction.

The primary objective of the VTG assay was to qualitatively determine whether vitellogenins were present in swordfish muscle tissue. A further objective, contingent upon positive results from the first, was to evaluate whether relative quantities of VTG could be differentiated between muscle tissues of male and female swordfish collected during spawning (March-August) and nonspawning (September-February) periods.

Results were incompletely successful. At a minimum, muscle tissue of adult female swordfish collected during the spawning season contained sufficient quantities of VTG for it to be detected using the tilapia assay. Further sampling and chemical assays would be required to quantify the effects of sex, maturity, and season on the amounts of VTG present in swordfish muscle tissue. To do so would require the development of an antibody assay, preferably monoclonal, which is specific to swordfish VTG. Development of a VTG assay specifically for swordfish is sought because at present it is our only likely means of identifying the sex of dressed swordfish carcasses, which provide most of the length-frequency data needed to monitor size composition of catch.

INTRODUCTION

Adult male and female swordfish have apparently different growth (Uchiyama et al., in press) and perhaps related (Pauly, 1979) natural mortality rates. Male and female swordfish also likely differ in catchability because of greatly different body size distributions: e.g., larger female swordfish are targeted by the Hawaii longline fishery (DiNardo and Kwok, in press). For these reasons, sex composition of the catch is needed as input to effective stock assessment and management of the fishery (Skillman, in press). Because swordfish are headed and gutted at sea, however, conventional methods of sex determination are not possible for landed fish, and the sex of dressed carcasses is not determinable using other criteria such as fin membrane coloration or the elongation of anal fin rays during the spawning season (R. Humphreys, unpubl. data). Unconventional biochemical techniques are required to sex market carcasses of swordfish.

One promising biochemical approach is screening carcasses for vitellogenin (VTG), a protein precursor to yolk secreted by the liver and present in the oocytes and other tissues of female fish and other egg-laying vertebrates (Lee et al., 1992; Lazier and MacKay, 1993; Specker and Sullivan, 1994; Folmar et al., 1995). The quantity as well as presence of VTG is important, because the males of some species can possess VTG-like substances (Kishida and Specker, 1993) and because the quantity of VTG in females varies with maturation and stage of the reproductive cycle (Specker and Sullivan, 1994). Screening market carcasses for relative amounts of VTG could in theory provide a sex-specific marker analogous to morphologically dissimilar (heterogametic) sex chromosomes. To date we do not know whether sex is genetically or environmentally determined in swordfish or in most other fishes, so determining sex from chromosomes, if possible, would require more expensive and time-consuming research than developing a sex-specific protein immunoassay.

METHODS AND MATERIALS

Market Sampling and Specimen Collection

Fully dressed (headed, eviscerated, and tailed) carcasses of swordfish were sampled at the Honolulu fish auction. On average, 10 days (estimated range 7-21 days) elapsed between capture by longline, cold (-2 to -3°C) storage aboard ship, and the time of off-loading at Honolulu. Fish were measured (eye-to-fork length, EFL, in cm), and sexed based on macroscopic appearance of gonads plus subsequent histological confirmation using a gonad specimen collected by a representative of the Hawaii Longline Fishery Observer Program during the heading and eviscerating procedure aboard ship. Gonad specimens were preserved in 4% formaldehydesea water for a minimum of 2 mo. Standard histological slides (hematoxylin stain, eosin counterstain) were then prepared and the slides viewed microscopically, using the histological criteria of Hunter and Macewicz (1985) to confirm shipboard determinations of sex. January-February 1995 collections were considered nonspawning season; specimens collected during May-June 1995 were classified as spawning season.

As each carcass was tailed at the auction, a ~ 25-g specimen of muscle tissue was removed by sterile scalpel from the caudal peduncle region. Each specimen was placed in an individual plastic bag and stored on ice for transport to the NMFS Honolulu Laboratory. Muscle specimens (both white and red muscle, plus attached connective tissue and skin) were trimmed and frozen at -20°C for 1-6 mo until shipment to the University of Rhode Island for biochemical analyses. Specimens were shipped (2-d transit) in a sealed container on dry ice.

Chemical Analyses

The basic premise tested was whether swordfish muscle tissue would react biochemically to a polyclonal antibody of vitellogenin (VTG), a glycolipophosphoprotein precursor to oocyte yolk, which had been developed previously for the percoid fish, tilapia (*Oreochromis mossambicus*), using an enzyme-linked immunosorbent assay (ELISA; Specker and Anderson, 1994). The first step, therefore, was to confirm that the existing assay (tilapia VTG antisera) still worked for muscle samples taken from known male and female tilapia.

Next, the extraction procedure was checked for swordfish tissue specimens: the effects of different buffers (e.g., salt concentrations, aprotonin, EDTA) were evaluated. The potential effect of white versus red muscle tissue (same fish) on VTG concentration was initially checked. Antisera were developed and screened for the highest affinity (reactivity or "binding") to the tilapia VTG antisera. Several swordfish specimens of known sex (both male and female) were then tested to confirm that bindings for male and female swordfish were different. Finally, extracts of swordfish tissue specimens of unknown sex were tested and evaluated using the tilapia VTG antiserum; sex was classified by magnitude of percentage binding and slope of serial dilutions of extract compared with the slope of the standard curve.

RESULTS AND DISCUSSION

Extraction Procedures

Extraction was complicated by the presence of skin and connective tissue in the muscle specimens provided for the assay. Muscle tissue had to be further separated from these other tissues, as connective tissue in particular made extracts gummy. Most tissue specimens included both red and white muscle, as it was initially suspected that these two types of muscle might contain different concentrations of VTG. The two muscle types did not differ, based on within-fish comparisons for two known-sex swordfish (one male and one female).

Antisera Affinity in Swordfish

Swordfish antisera to the polyclonal antibodies developed against tilapia VTG bound with varying affinities because of several factors. Binding affinity differed at least qualitatively for samples of male and female swordfish. Serial dilution of extracts from known males did not displace tilapia VTG, but serial dilutions of known female extracts did displace tilapia VTG in a dose-dependent manner (Fig. 1).

Screening the reactivities of swordfish tissue unknowns was not completely successful. Reactivities of swordfish muscle tissue varied greatly among fish of the same sex. As a result, one-third (males) and more than one-half (females) of the sex determinations based on histology were misclassified by the ELISA assay, even though the cross-classification was not statistically independent (2 x 2 Contingency χ^2 test, $0.05 > P > 0.25$; Zar, 1984, p. 62; Table 1). This, in part, might have reflected the insensitivity of the swordfish antisera to tilapia VTG. It is reasonable that cross-reactivities between swordfish and tilapia VTGs would be weak, given the diversity of vitellogenins present in perciform fishes (Lee et al., 1992).

Sex effects on reactivities were likely further obscured by body size and time of year. Same-sex fish (confirmed by histology to include immatures through large mature adults) varied greatly in body size and season of collection (including spawning and nonspawning seasons). Both maturation and reproductive state are known to influence VTG concentration (Specker and Sullivan, 1994). Hence, larger sample sizes would be required to simultaneously quantify the effects of maturation (immature, mature) and reproductive activity (spawning, nonspawning) for males and females, after a direct assay for swordfish VTG has been developed.

CONCLUSIONS AND RECOMMENDATIONS

Results of these initial tests of unknown-sex swordfish tissues suggest that swordfish muscle tissue contains VTG (or VTG-like lipoproteins) against which antisera could be developed and used in an immunoassay to identify the sex of individual fish. The assay would have to be developed specifically for swordfish in order to increase the antiserum binding affinity and sensitivity of the test. Thus, swordfish VTG would have to be purified from swordfish plasma. The best approach to take would be to develop both specific monoclonal (mAB) and polyclonal antibodies to swordfish VTG. Each approach has its positive and negative aspects. Developing a specific mAB might be more difficult or costly in the short term but could produce an assay which would provide the parent culture necessary to generate all subsequent productions of the assay. Polyclonal antibodies might initially be easier to produce but would require that the quantity of assay initially produced be sufficient for all subsequent applications of the assay. A specific assay would have to be developed to identify the sex of swordfish carcasses with acceptable accuracy for stock assessment needs. Once successfully developed, the assay would have to be applied on a production level to screen large numbers of swordfish carcasses at auction on a continuing, long-term basis.

A recent study by Heppel et al. (1995) indicates that a mAB to a generic section of the teleost VTG molecule has already been developed. Arrangements are presently being made to evaluate the suitability of this assay as one potentially more sensitive approach to screening the sex of dressed swordfish carcasses.

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Table 1.--Results of sex determinations of swordfish (*Xiphias gladius*) market carcasses based on the results of VTG (ELISA) assays of caudal peduncle muscle tissue versus histological examination of gonad tissue. Period: S = Spawning; NS = NonSpawning. An * flags an incorrect assay determination; a (?) denotes an uncertain assay; a ✓ indicates a specimen of known sex used for assay calibration.

Specimen number	Day-Mo-Yr collected	Period (S,NS)	EFL in cm	Sex as determined by: ELISA assay	Sex as determined by: Histology
LEV-171	05Jan95	NS	104	female	female
LEV-175	11Jan95	NS	203	female	female
LEV-177	11Jan95	NS	190	female	female
LEV-179	11Jan95	NS	182	female	female
LEV-194	12Jan95	NS	209	* male	female
LEV-258	16Jan95	NS	220	female	female
JED-086	05Feb95	NS	165	* male	female
JED-088	05Feb95	NS	164	* male	female
ADP-365	13Feb95	NS	215	* male	female
ADP-386	14Feb95	NS	184	male	male
ADP-390	14Feb95	NS	196	male	male
ADP-396	14Feb95	NS	219	male	male
ADP-407	15Feb95	NS	196	* female(?)	male
ADP-412	15Feb95	NS	203	* male(?)	female
ADP-431	17Feb95	NS	181	male	male
ADP-435	18Feb95	NS	172	male	male
ADP-439	18Feb95	NS	189	male	male
ADP-455	19Feb95	NS	191	female	female
ADP-462	19Feb95	NS	180	male	male
ADP-471	20Feb95	NS	127	(fe)male(?)	male
ADP-475	20Feb95	NS	138	* male	female
ADP-479	20Feb95	NS	211	* female	male
ADP-488	21Feb95	NS	168	male	male
ADP-491	21Feb95	NS	185	* female	male
ADP-508	23Feb95	NS	203	* female(?)	female
JED-322	13May95	S	212	female	female ✓
JED-323	16May95	S	118	male	[lost]
JED-335	19May95	S	127	* male	female
JED-340	20May95	S	133	* male	female
JED-342	20May95	S	132	* female	male
JED-344	21May95	S	127	male	male
JED-346	21May95	S	134	male	male
JED-349	22May95	S	198	female(?)	female ✓
LEV-515	05Jun95	S	221	* male	female
LEV-524	06Jun95	S	125	male	male
LEV-525	06Jun95	S	166	female	female
LEV-528	06Jun95	S	126	male	male
LEV-529	06Jun95	S	151	male	male ✓
LEV-531	07Jun95	S	148	female	female
LEV-535	08Jun95	S	131	male	male ✓

Table 1.--Continued.

Specimen number	Day-Mo-Yr collected	Period (S,NS)	EFL in cm	Sex as determined by	
				ELISA assay	Histology
LEV-540	09Jun95	S	137	* female(?)	male
LEV-542	09Jun95	S	149	female	female
LEV-546	11Jun95	S	158	male(?)	male
LEV-563	12Jun95	S	161	male	male
LEV-569	13Jun95	S	125	male	male
LEV-571	13Jun95	S	163	female	female ✓
LEV-577	13Jun95	S	167	* female	male ✓
LEV-582	15Jun95	S	115	* female	male
LEV-583	15Jun95	S	147	* female(?)	male
LEV-587	16Jun95	S	162	* female	male ✓

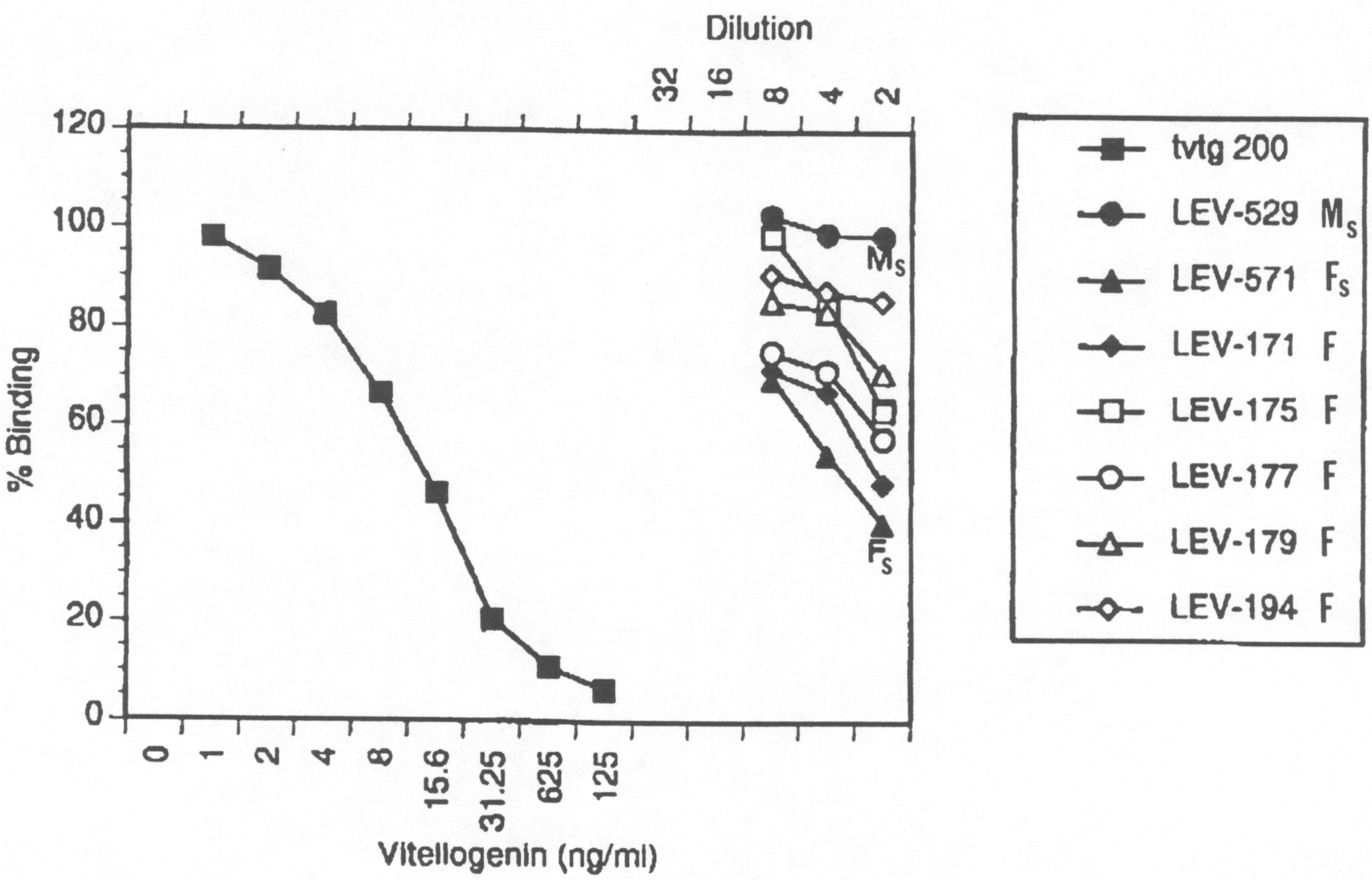

Figure 1.--Plots of percentage binding versus concentration of tilapia (*Oreochromis mossambicus*) VTG antisera for known-sex muscle tissue specimens of tilapia and swordfish (*Xiphias gladius*, two spawning fish--one male and one female--and five pre-spawning fish of known female sex).